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Studies on the host–parasite relationship in *Schistosoma mansoni*-infected mice: the immunological dependence of parasite egg excretion

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Summary. CBA mice deprived of their T cells by means of thymectomy and anti-thymocyte serum and subsequently infected with *Schistosoma mansoni* were found to have substantially fewer parasite eggs in their faeces than similarly infected immunologically-intact control animals. The number of parasite eggs deposited in the tissues of T-cell deprived mice was by comparison only marginally lower than in control mice. Administration of serum obtained from normal mice with chronic *S. mansoni* infections partially restored the egg excretion rate in infected deprived mice, and also resulted in an increased number of eggs being deposited in the liver and intestine of these animals.

INTRODUCTION

During routine parasitological observations made on T-cell deprived and immunologically-intact mice infected with *Schistosoma mansoni* it was found that, compared with the controls, the former animals had markedly fewer parasite eggs in their faeces. We here report our preliminary results of this phenomenon, and suggest that this parasite may make considerable use of the immune responses of the definitive host for the continued transmission of the disease.

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MATERIALS AND METHODS

Mice

Male CBA/Lac mice were used throughout except as a source of thymocytes for preparation of rabbit anti-mouse thymocyte serum and for maintenance of the parasite (see below).

Method of T-cell deprivation

Four week-old mice were thymectomized by the method of Law, Bradley & Rose (1963), and received 4 injections of 0·25 ml rabbit anti-mouse thymocyte antiserum (ATS) on alternate days within 10 days of the operation. A period of between 30 and 60 days was allowed between the administration of the ATS and infection with *Schistosoma mansoni*.

ATS was prepared according to the method of Levey & Medawar (1966). Thus, a cell suspension was prepared from the thymuses of 4 week-old random-bred T.O. mice and after washing three times in Medium 199 (Burroughs Wellcome), it was injected intravenously into 2 kg albino rabbits (thymuses from ten donor mice/rabbit). The thymocyte injection was repeated after a fortnight, and

the rabbits exsanguinated one week after the second injection. The antiserum was heat-inactivated at 56° for 30 min and stored at -20° until use. Serum from at least three rabbits was pooled in the preparation of any batch of ATS.

Unpublished evidence that thymectomy and administration of ATS is an effective means of inducing a state of relatively permanent immunodepression in mice is as follows: (1) ATS-deprived mice retained AKR skin grafts for a median time of 46 days, compared with a rejection time of 18 days on intact mice. Four deprived mice out of a group of nine retained their skin grafts for over a year; (2) the mean number of indirect (anti-mouse IgG-developed) haemolytic plaque forming cells (Cunningham & Szenberg, 1968) detected per million nucleated cells in the spleens of ATS-deprived mice 7 days after injection of 5×10^8 sheep erythrocytes was 12 compared with 1740 found in the spleens of control mice; (3) fewer than 10% of the normal number of PHA-responsive lymphocytes were detected in the blood of deprived mice, as determined by the method described in Doenhoff, Janossy & Kerbel (1976).

Immunologically-intact control mice were always of the same age as the deprived animals.

Parasite

A Puerto Rican strain of *S. mansoni* was maintained by laboratory passage in *Biomphlaria glabrata* snails, and random bred T.O. strain mice, (Taylor, Amin & Nelson, 1969). Infected snails were phototropically induced to shed cercariae into dechlorinated water, and the larvae used for infection within 3 h of emergence (Olivier, 1966).

Method of infecting and perfusing mice

Percutaneous infections and portal system perfusions were performed according to the method of Smithers & Terry (1965).

Faecal egg counts

Mice were placed individually in 400 ml plastic beakers and allowed to defaecate. Single faecal pellets of between 10 and 40 mg were weighed to the nearest milligram and placed in isotonic saline solution. After approximately 30 min the pellets were disrupted by aspiration in a 10 ml plastic syringe without needle, and the larger undigested food particles removed by filtration through a 320μ metal sieve. Each filtrate was passed through a Whatman No. 4 filter paper and the eggs retained on the paper were stained with saturated Ninhydrin solution as described by Bell (1963). Dried papers were examined at a magnification of $\times$ 32. Results are expressed as the number of eggs/100 mg faecal matter or $\log_{10}$ (x+1) number of eggs/100 mg. In instances in which groups with high and low faecal egg counts were being compared no obvious difference was noted between the experimental and control animals in terms of the rate of defaecation.

Tissue egg counts

Following perfusion of infected mice, the large intestine, caecum, small intestine and the liver, (the latter always excluding the two lobes encompassing the gall bladder, these having been sometimes taken for histological examination) were removed and stored at $-20°$ until required. The gut and liver were digested in 20 ml of 5% potassium hydroxide solution at 37° for 16 h (Cheever, 1968). Fifty μl aliquots of the digests were placed under coverslips on microscope slides and the eggs counted at $\times$32 magnification. Each digest was examined in triplicate and the mean results expressed as eggs/tissue, eggs/worm pair/tissue, eggs/mouse or eggs/worm pair/mouse. (one worm pair=the number of male or female worms, whichever was the lower, found in the perfuseate). No correction has here been made for the eggs that were present in the portion of liver removed for histology, this being approximately 35% by weight of the whole organ.

Chronic Infection serum (CIS)

CBA mice were percutaneously infected with twenty five *S. mansoni* cercariae. Approximately 12 weeks later those mice in which no eggs were detected in the faeces, and which gave no immunodiffusion precipitin lines with egg antigens (Ouchterlony, 1958; Pelley, Pelley, Hamburger, Peters & Warren, 1977) were rejected. The remainder were bled twice weekly from the retro-orbital venous plexus (0·3–0·4 ml blood/mouse/bleeding) until the survivors were finally exsanguinated. The serum was stored at $-20°$ and on the first day that it was required for use, all that had been obtained from one batch of mice was thawed and pooled. The remainder of the pool was refrozen in aliquots suitable for use on succeeding days. Normal mouse serum (NMS) was obtained from uninfected CBA mice and stored in a similar manner.

RESULTS

Figure 1 gives the results of the number of parasite eggs detected in the faeces of normal and T-cell deprived animals infected with thirty five or 175 *S. mansoni* cercariae. As might be expected, the more heavily infected animals have a higher egg excretion rate, but fewer eggs/100 mg faecal matter were detected in both groups of deprived mice than in the respective immunologically-intact groups throughout the time course. The mean number of eggs/100 mg detected in the faeces of the two types of mice was significantly different from day 45 to day 55 after infection with thirty five cercariae, ($P < 0{\cdot}02$, Student t test,) and on days 41 and 45 after infection with 175 cercariae ($P < 0{\cdot}001$).

The day 45 post-infection faecal egg count and the worm and tissue egg burdens of the more heavily infected mice in the first experiment (Fig. 1) are given in the first two rows of Table 1, as are similar determinations made on normal and T-cell deprived animals in three other experiments. No consistent differences between the two types of mice were found in the perfuseable worm burdens, though the number of eggs/worm pair deposited in the tissues of the deprived mice was in general lower than in the normal controls, the reduction being by as much as 50% (e.g. Exp. 2, Table 1). However, the most dramatic difference in the host-parasite relationship in all four experiments was in terms of faecal egg counts, deprived mice excreting between approximately five times (Exp. 1) and 70 times (Ex. 3) fewer eggs than normal mice.

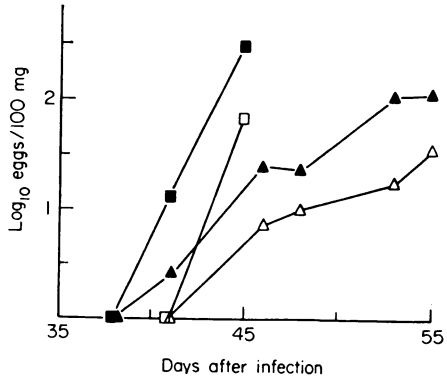

Figure 1. The $\log_{10}$ (x+1) mean number of eggs detected in the faeces of normal (closed symbols) or T-cell deprived (open symbols) mice infected with 35 (triangles) or 175 (squares) *S. mansoni* cercariae. Ten mice were studied in each of the 4 groups.

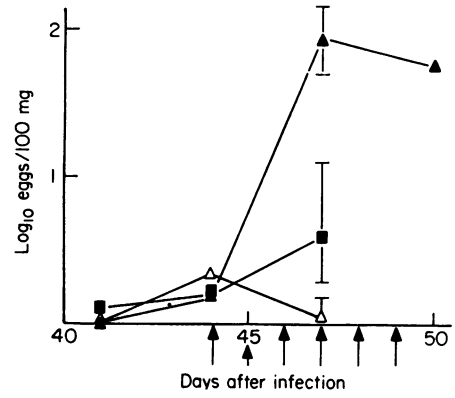

Figure 2. The $\log_{10}$ (x+1) mean number of eggs/100 mg faecal matter found in deprived mice infected with 200 *S. mansoni* cercariae for 44 days before the commencement of daily intraperitoneal injections (arrows) of 0·7 ml chronic infection serum (▲), normal mouse serum (△) or isotonic saline (■). A minimum of seven mice was examined in each of the three groups. Variability on day 47 is indicated by ± 1 Standard Deviation.

When *S. mansoni*-infected T-cell deprived mice were injected intraperitoneally daily with 0·7 ml/day CIS from days 44 to 49 (inclusive) after infection with 200 cercariae the rate of egg excretion increased markedly, whereas injection of control groups with the same volume of saline or NMS had little effect (Fig. 2). A second batch of CIS prepared from different donors from that used in Fig. 2 yielded similar results which are shown in Fig. 3. In neither of these two experiments, in which the mice were infected with 200 cercariae, was the rate of egg excretion in the CIS-injected mice restored to the level in immunologically-intact mice infected with 175 cercariae (Fig. 1). Nevertheless, there was about a tenfold difference in faecal egg counts between the CIS-injected and the control animals in both experiments.

The injection of CIS in each of the experiments depicted in Figs 2 and 3 had some effect on the number of parasite eggs found in the murine host tissues (Table 2). Thus, in both experiments the eggs/worm pair found in the liver and intestine of the CIS-treated groups was marginally greater than in control groups.

When CIS was injected daily into immunologically-intact *S. mansoni*-infected mice commencing about 5

Table 1. The number of eggs detected in the faeces of normal and deprived *S. mansoni*-infected mice, and the tissue egg and perfuseable worm burdens of the same mice. A time course of egg excretion rate for Exp. 1 is given in Fig. 1. Experiments 2–4 were performed at different times with different batches of infective material. All faecal egg determinations were performed on day 45 post-infection. Variability is indicated by ±1 standard deviation

Exp.	Type of mouse	No. of cercariae in infection	No. of mice in faecal sample	Faecal egg count	Day of perfusion	No. of mice perfused	No. of worms recovered	No. of worm pairs recovered	No. of eggs found in liver	No. of eggs/wm pr. in liver	No. of eggs found in gut	No of. eggs/wm pr. in gut	Total no of eggs	Total no. of eggs/wm pr.
1	Normal	175	9	303·2 ± 98·8	45	9	76·7 ±20·7	33·7 ±10·0	19318 ± 5723	584 ±144	46844 ±14144	1398 ± 166	66163 ±17857	1982 ± 188
	Deprived	175	9	64·1 ± 57·1	45	9	64·4 ±21·7	27·3 ±10·1	12741 ± 6149	453 ±129	31052 ±14693	1107 ± 271	43793 ±20573	1560 ± 385
2	Normal	150	10	314·2 ±191·8	46	10	66·5 ±28·9	30·6 ±13·5	20160 ± 7014	813 ±515.9	54067 ±14178	2267 ±1554	74227 ±20693	3080 ±2066
	Deprived	150	11	5·0 ± 8·6	46	11	69·4 ±17·2	33·0 ± 8·7	13164 ± 3548	415 ±119·5	34703 ±12375	1077 ± 324	47867 ±15179	1492 ± 416
3	Normal	200	7	224·3 ±136·1	46	10	59·6 ±19·9	26·1 ±10·6	21907 ± 7898	899 ±278	54947 ±21776	2276 ± 860	76853 ±29003	3174 ±1087
	Deprived	200	9	3·3 ± 3·2	46	9	85·1 ±14·8	37·2 ± 7·9	27763 ±16271	737 ±429	48459 ±15066	1321 ± 413	76222 ±25000	2059 ± 606
4	Normal	200	12	309·6 ±241	48	5	86·6 ±11·4	38·0 ± 4·6	29173 ± 6185	764 ± 96·5	86133 ±12367	2271 ± 202	115307 ±18080	3035 ± 268
	Deprived	200	10	5·7 ± 11·3	48	5	85·4 ±10·9	35·8 ± 3·1	22480 ± 4611	627 ±103	67013 ±11088	1862 ± 146·9	89493 ±14167	2488 ± 194

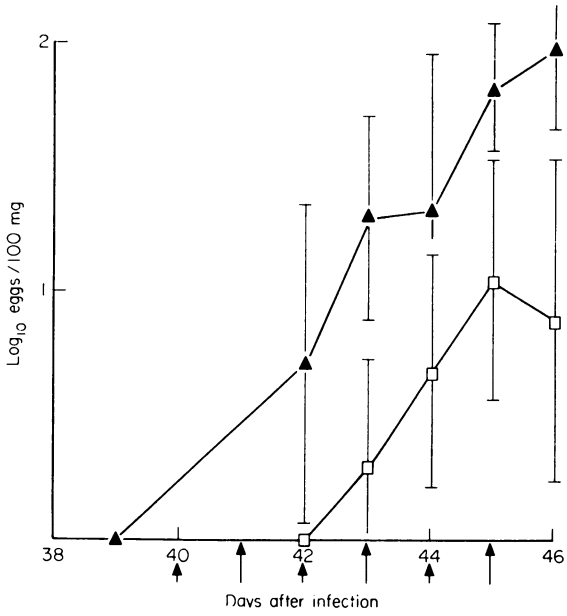

Figure 3. The $\log_{10}$ (x +1) mean number of eggs detected in the faeces of T-cell deprived mice infected with 200 *S. mansoni* cercariae and injected (arrows) intraperitoneally with 0·7 ml/day CIS (▲), or N.M.S. (□). Seven mice were studied in each group. Variability about the mean indicated by ± 1 standard deviation.

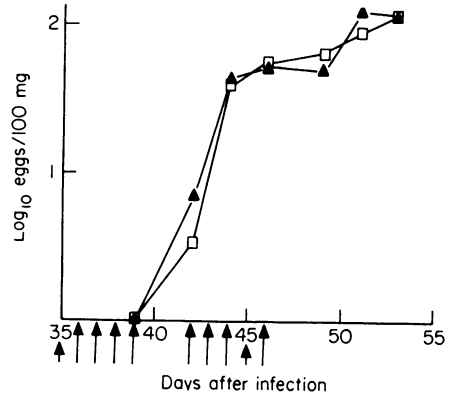

Figure 4. The $\log_{10}$ (x +1) number of eggs/100 mg faecal matter detected in the faeces of immunologically intact mice infected with 100 *S. mansoni* cercariae and injected (arrows) with 0·7 ml/day CIS (▲) or with the same volume of NMS (□). On perfusion of both groups 53 days after infection the NMS-treated group (n=8) was found to have a mean of 11·0±4·6 worm pairs and 1147±555 eggs/worm pair in the liver; the group treated with CIS (n=7) had a mean of 9·4±2·3 worm pairs and 1014±227 eggs/worm pair in the liver.

days before parasite eggs are first detected in the faeces, but at the dosage used for the deprived animals above, no difference was observed in the rate of egg excretion (Fig. 4), or in the worm or tissue egg burdens when compared with NMS-treated similarly infected controls (Legend to Fig. 4).

DISCUSSION

Although T-cell deficient mice have previously been infected with *S. mansoni* (Fine, Buchanan & Colley, 1973; Buchanan, Fine & Colley, 1973; Hsü & Hsü, 1976; Phillips, Di Conza, Gold & Reid, 1977) faecal egg determinations appear not to have been performed in these studies. Newsome (1963) found that corticosteroid treatment of infected baboons in some instances resulted in a reduced faecal egg output, and this was perhaps wrongly interpreted by

Table 2. The mean worm burdens and the mean number of tissue eggs/worm pair found in groups of T-cell deprived mice infected with *S. mansoni* cercariae and treated with 0·7 ml/day chronic infection serum. Control mice in both experiments were given the same volume of normal mouse serum at the same times.

Mice in exp. 1 were given serum from the same donors and at the same times after infection as the mice in Fig. 2, but were perfused separately. Results for exp. 2 are derived from the same animals as were used for Fig. 3. Variability about the mean is indicated by ±1 standard deviation

Exp.	Deprived mice	Day of perfusion	No. of mice	No. of worm pairs	Eggs/wm pair liver	Eggs/wm pair intestine
1	NMS-treated	47	5	36·6± 6·5	568± 89	1824± 187
	CIS-treated		6	36·3±15·1	980±479	2175±1204
2	NMS-treated	46	7	25·9± 6·3	616±103	1449± 422
	CIS-treated		7	25·0± 6·0	825±120	1765± 441

Newsome (1963) as a schistosomicidal effect of the drug (see Coker, 1957; Weinmann & Hunter, 1960). We have, however, also observed a reduction in the rate of egg excretion following treatment of infected mice with hydrocortisone acetate, whereas the number of worm pairs and eggs/worm pair deposited in the liver was seemingly unaffected (Musallam, unpublished results).

In view of the observations that T-cell deprived mice have lower egg excretion rates than intact animals, and CIS can partially restore faecal egg counts whereas normal mouse serum cannot, it is for the moment being assumed that an immune effector mechanism is lacking in the serum of deprived animals, and that when present, it acts directly or indirectly to allow parasite eggs to successfully traverse the intestinal wall into the lumen. It is, however, possible that some non-immunological serum-borne factor is lacking in infected deprived mice. Thus, for example, the granulomatous inflammatory reactions normally associated with eggs that are deposited in the tissues of normal animals (Warren, 1976) may produce and release soluble pharmacologically active substances required for egg production and excretion. The relative absence of inflammatory activity around the *S. mansoni* eggs in T-cell deprived mice may result in a shortage of such factors. Although antibody is the most obvious candidate for a serum-borne immunological effector mechanism, there is no direct evidence of antibody involvement in the experiments described here. Experiments are in progress to define more accurately the active component in serum.

Should the serum factor be identified as antibody, it will remain to be determined which antigen is responsible for inducing the synthesis of that antibody and which is its target during the egg excretion process. Identification of the antigen inducing the synthesis of the particular antibody should be possible using more defined stages of the parasitic life cycle to infect the serum donors. Thus, immune sera are at present being raised against cercarial, schistosomular, adult worm and egg antigens by sensitizing mice respectively with irradiation-attenuated larvae, infections of male or female worms alone, and isolated eggs alone. In a preliminary (unpublished) experiment it has been found that seven daily injections of serum from a rabbit that had been immunized with *S. mansoni* egg homogenate raised the faecal egg count of *S. mansoni*-infected deprived mice from a mean of $1{\cdot}74\pm3{\cdot}9$/100 mg to $37{\cdot}5\pm46{\cdot}5$/100 mg faecal matter, indicating that parasite eggs may indeed be important in inducing synthesis of the relevant serum component.

The target of the antibody, if such it is, may be more difficult to elucidate. Injections of CIS into deprived mice appeared to have some enhancing effect on the number of eggs deposited in the tissues (Table 2) and it is possible that this marginal effect was all that was required to increase egg density (or egg pressure) at the site of their production in the mesentery sufficiently to facilitate their migration through the gut wall. Such a hypothesis supposes that antibody acts on *S. mansoni* worms in a manner which increases their fecundity.

An additional or alternative action of serum antibody may be directed towards the egg. Proteolytic enzyme activity has been found in *S. mansoni* eggs (Kloetzel, 1967; 1968), and the eggs produced by worms infecting T-cell deprived mice may be lacking in such enzymes or the enzymes may in some way be defective or inactive in the absence of an immune response generated by the host. Thus it can be envisaged that the synthesis or activity of the enzyme(s) responsible for breaking down intestinal tissue to allow passage of the egg to the lumen might be induced by specific interaction with antibody. *S. mansoni* eggs isolated from the tissues of infected normal and T-cell deprived mice are being compared with each other with respect to hypotheses such as these.

It is perhaps paradoxical in terms of the above hypotheses that injections of CIS into normal mice during the time that the number of eggs was building up in the tissues should have had no marked effect on egg excretion, either in terms of it being initiated earlier or proceeding at a faster rate (Fig. 4). The serum that was injected into the normal recipients in this experiment, as in the experiments with deprived mice, was taken from mice that had been infected for at least 12 weeks, and the donors would therefore have had at least 7 weeks more immunological experience of the infection than the recipients. As has been noted previously by Weinmann & Hunter (1961), injections of serum from infected mice had no marked effect on worm burden or on the number of eggs in liver tissue of the recipients (Legend to Fig. 4).

It is tempting to speculate that the apparent immune-dependence of schistosome egg excretion

demonstrated in the model system used here may also be relevant to other schistosome species and other definitive hosts, particularly human. Our preliminary observations may only be supported by epidemiological evidence when attempts are made to correlate human egg excretion data directly with immune status, with some independent test being used to determine parasite worm burden. In any case, schistosome-infected patients with an immune-deficiency as severe as that found in thymectomized, ATS-treated mice are likely to be found only rarely.

The interpretation by Warren (1973), of Rao's (1933) paper on *Schistosoma nasalis* suggests that in this species of schistosome also the immune response may play an active part in the excretion of eggs. Furthermore, a number of protozoan infections of rodents, particularly those strains which seem to be the most virulent, appear to be unable to parasitize immunosuppressed hosts to the same extent as intact hosts. Thus neonatal thymectomy (Wright, 1968) and ATS-treatment (Wright, Masembe & Bazira, 1971) of hamsters, and ATS-treatment of mice (Sheagren & Monaco, 1969) induced a prolongation of life of the host after *Plasmodium berghei* infections, and *Trypansoma brucei*-infected deprived mice have been found to live somewhat longer than infected intact controls (Terry & Hudson; Cooper, unpublished results). It may therefore be expected that further instances will be described of parasites which not only become adapted to survival for long periods in what should be immunologically hostile environments, but which have further evolved to make active use of immune effector mechanisms for the purposes of that survival.

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